

## MYOCARDIAL BETA ADRENERGIC RECEPTOR RESPONSE COUPLING IN RATS WITH CHRONIC RENAL FAILURE

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### Summary

To study hormone receptor response coupling in the cardiovascular system in uraemia, beta adrenergic receptor density and adenylyl cyclase activity were measured in the ventricle of chronically uraemic rats.  $^3\text{H-DHA}$  binding sites were decreased by 20 per cent in the myocardium without any change in the dissociation constant. Hormone stimulated adenylyl cyclase activities (ACA) were also decreased by 30–45 per cent in uraemic ventricles. These results indicate that both receptor and post-receptor defects are involved in the poor responsiveness to beta adrenergic stimulation by the heart in uraemic rats.

### Introduction

It has been previously reported that the cardiovascular response to catecholamine is reduced both in patients [1,2] with chronic renal failure and in uraemic animals [3,4]. Some investigators explained this low physiological responsiveness to a decreased cardiac beta adrenergic receptor density or decreased myocardial adenylyl cyclase activity in acutely uraemic rats [5]. Very little is known about adrenergic receptor response coupling in chronic uraemia. This experiment was designed to study the possible biochemical mechanism of receptor cyclase coupling in the myocardium of uraemic rats.

### Method

*Animal models* Male Sprague Dawley rats aged 10–11 weeks weighing 300g were used. Renal failure was induced by freezing the renal parenchyma by cryosurgery followed by contralateral nephrectomy two weeks later. The systolic blood pressure was measured every two weeks by the tail cuff method and the blood urea nitrogen and serum creatinine measured. The rats which developed hypertension  $>160\text{mmHg}$  during the eight weeks from induction of chronic renal failure and cardiac hypertrophy with a ventricular weight/body weight

ratio of more than 2.2 were excluded for this experiment. In addition to these criteria only the rats with serum creatinine between 1.8–3.5mg/dl were used. Approximately a half of all operated rats met these criteria.

**Assay method** Myocardial beta adrenergic receptors and adenylyl cyclase activities were studied by the methods previously reported [6]. Briefly, after cervical dislocation left ventricles were rapidly removed, perfused with cold saline and frozen in liquid nitrogen; they were kept in a deep freeze until assay. The total homogenate passed through four layers of gauze was used for radioenzymatic assay of adenylyl cyclase. Enzyme activities were studied following the addition of varying concentrations of isoproterenol (iso), glucagon (glu), guanosine 5'-triphosphate (GTP), guanosine 5'-imidotriphosphate [Gpp(NH)p], NaF and forskolin. Beta adrenergic receptor was studied using <sup>3</sup>H-dihydroalprenolol (DHA) on the 40,000 G pellet.

## Results

**Laboratory findings** Blood chemistry at the time of sacrifice (8–9 weeks after induction of chronic renal failure) showed profound uraemia (Table I). Nor-epinephrine and glucagon concentrations, obtained by an indwelling arterial

TABLE I. Laboratory findings of sham control and chronic renal failure rats. Values are mean  $\pm$  SEM. Hormones were measured in eight animals in each group

	Creatinine mg/dl	BUN mg/dl	Ca mg/dl	PO <sub>4</sub> mg/dl	Epi pg/ml	Norepi pg/ml	Glucagon pg/ml
Control (12)	0.6 $\pm 0.03$	14 $\pm 0.9$	9.6 $\pm 0.14$	7.1 $\pm 0.10$	44.2 $\pm 4.70$	200 $\pm 46.3$	110 $\pm 13.3$
CRF (16)	2.7* $\pm 0.10$	148* $\pm 7.7$	8.7 $\pm 0.17$	10.8* $\pm 0.60$	49.0 $\pm 7.36$	350* $\pm 55.8$	168* $\pm 17.7$

\* Significant difference from sham ( $p < 0.05$ )

catheter in awake and unrestrained animals, were significantly increased in the chronic renal failure group. Systolic blood pressure did not differ between normal and chronic renal failure rats. Left ventricular weight was not changed, but the heart weight/body weight ratio was increased significantly due to some loss in body weight (Figure 1).

**<sup>3</sup>H-dihydroalprenolol binding** Protein concentration of the myocardial homogenate and 40,000G pellet did not differ between control and chronic renal failure group (data are not shown). This might indicate that water content and membrane recovery did not differ between them. <sup>3</sup>H-DHA binding in chronic renal failure rats was decreased significantly whether expressed as fmol/mg protein or pmol/ventricle without any change in dissociation constant (Kd) (Table II).

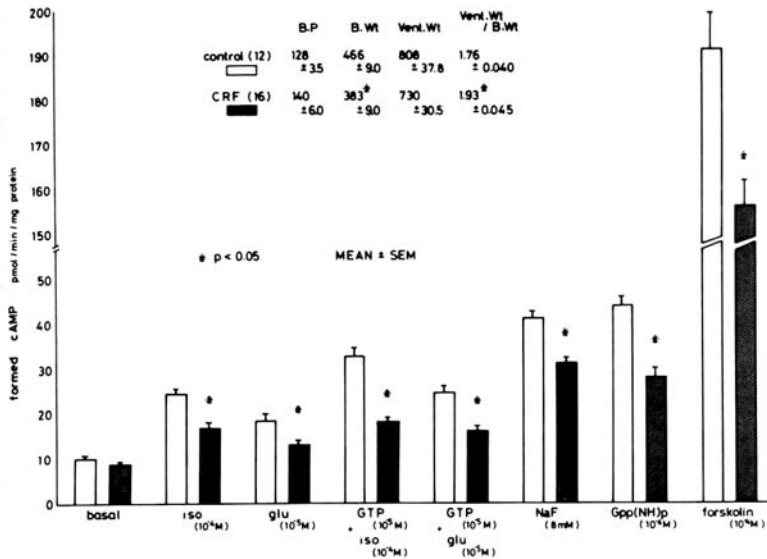


Figure 1. Body weight, left ventricular weight and adeny cyclase activities of 12 normal and 16 chronic renal failure rats

TABLE II. <sup>3</sup>H-dihydroalprenolol binding to rat myocardial membrane. Data are shown as mean ± SEM

	fmol mg protein	pmol g ventricle	pmol ventricle	Kd (nM)
Control (10)	39.3 ±2.5	3.2 ±0.2	2.8 ±0.2	3.1 ±0.3
CRF (12)	31.2* ±1.9	2.6* ±0.1	1.9* ±0.1	4.2 ±0.3

\* Significant difference from control

*Adenyl cyclase activity* Uraemic myocardium had significant decreases in isoproterenol or glucagon-stimulated activities as well as NaF-, Gpp(NH)p- and forskolin-stimulated activities, although there was no change in basal activity (Figure 1). Dose-response curves of isoproterenol and glucagon stimulated adenyl cyclase activity in uraemia showed a significant decrease in  $V_{max}$  (maximal velocity) with no change in  $EC_{50}$  (concentration of iso or glu required for half maximal response) (Figure 2).

## Discussion

Many previous studies have reported autonomic dysfunction of the cardiovascular system in uraemic patients and experimental animals. The genesis of this

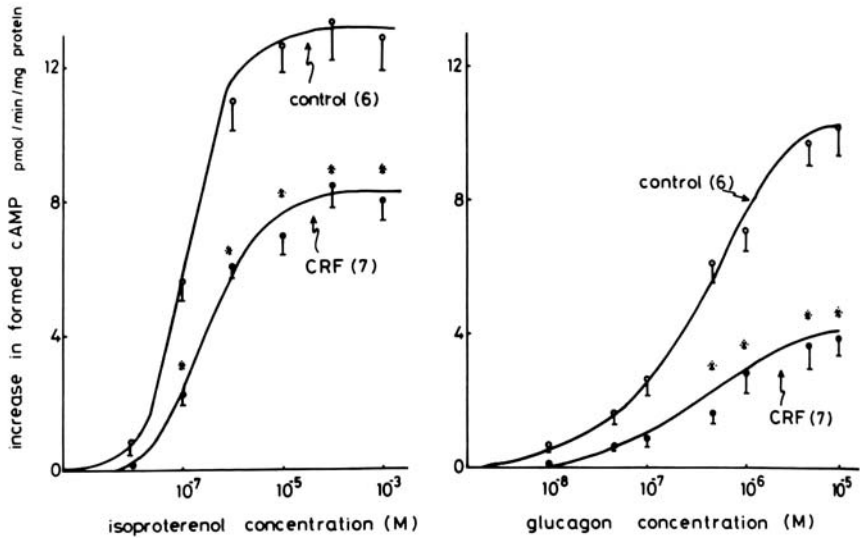


Figure 2. Dose response curves of isoproterenol-stimulated (left) and glucagon-stimulated (right) adenylyl cyclase activities. Results are mean and SEM. Parentheses indicate the number of experiments. EC50 calculated by probit analysis showed no difference between the two groups

abnormality seems to be multifactorial. Chronic uraemia is often associated with cardiac hypertrophy due to long-standing hypertension or a hyperdynamic circulation, which is well known to have low responsiveness to adrenergic stimulation. In this present study criteria were used to exclude the factors of systemic hypertension and cardiac hypertrophy.

It is generally accepted that hormone sensitive adenylyl cyclase consists of at least three functional units: a specific hormone receptor at which beta adrenergic agonist or other hormones act, a guanine nucleotide binding regulatory component (G/F) at which GTP, Gpp(NH)p or NaF acts, and a catalytic compartment at which forskolin acts directly [7]. Our present results indicate that uraemic myocardium seems to have at least two distinct impairments in the beta adrenergic receptor response coupling system, which has been reported in the acute uraemic rat ventricle by Mann et al [5]. One is the low density of beta adrenergic receptors. The other biochemical defect(s) lies in the catalytic unit of adenylyl cyclase or both in G/F and the catalytic unit, which remains to be clarified by further studies.

It is also interesting that uraemic ventricles seem to have defects not only specific to beta adrenergic stimulation, but also to glucagon. Glucagon receptors were not measured in this experiment, but Dighe et al [8] reported a decreased number of glucagon receptors with decreased cyclase activity in uraemic rat liver. Desensitization can possibly explain these defects in receptor and cyclase systems, because our study demonstrated significant increase in plasma glucagon and norepinephrine in the uraemic rats. Our previous study also showed that

chronic infusion of epinephrine for two weeks led not only to decreased beta receptors and isoproterenol-stimulated adenylyl cyclase activity, but also to decreases in glucagon-stimulated NaF-, Gpp(NH)p- and forskolin-stimulated activity [8]. Parathyroid hormone itself or by enhanced prostaglandin synthesis may possibly cause heterologous desensitization to adrenergic hormone and glucagon. Both intact parathyroid hormone and its amino terminal fragments are known to blunt the pressure effects of norepinephrine. Massry's group [10] have shown that parathyroidectomy in rats with chronic renal failure is followed by a return to normal in the pressure response to norepinephrine.

Our recent study showed that isoproterenol stimulated adenylyl cyclase activity in the pellet from uraemic rat myocardium was improved to some extent by reconstitution either with cytosol of a normal rat ventricle or with dialysed cytosol of uraemic rats. This may indicate that relatively small molecular uraemic substances participate to some extent in inhibition of hormone stimulated adenylyl cyclase activity.

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